



Original Research Article

Factors affecting stability of lycopene and β -carotene from Gac aril powder by freeze drying

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ABSTRACT

Gac fruit (*Momordica cochinchinensis* (Lour.) Spreng.) is originated in the area of East and Southeast Asia. Gac fruit is a rich source of carotenoid and antioxidant compounds. However, carotenoid compounds can be decomposed easily when exposed to heat, light and oxygen. The aim of this research focused on factors affecting stability of lycopene and β -carotene from freeze-dried Gac aril powder. For freeze drying, a factor varied concentrations of maltodextrin DE 10 per fresh Gac aril (0%, 5%, 10% and 15% w/w). Quality of freeze-dried Gac aril powder was further determined at different storage time (0, 5, 10, 15, 20, 25 and 30 days) and temperature (4°C and room temperature (27°C-30°C)). Lycopene, β -carotene and total phenolic content, antioxidant activity (DPPH assay) and color of Gac aril powder were evaluated. The results showed that the optimal condition of maltodextrin addition was at 0%. The physicochemical properties of 0% maltodextrin added Gac aril powder at 4°C stored up to 20 days were not significant ($p \leq 0.05$). The shelf life of freeze-dried product was 20 days at 4°C having the lycopene content of 0.99 ± 0.02 (mg/100g of dry weight), β -carotene content of 0.52 ± 0.04 (mg/100 g of dry weight), antioxidant activity content of $44.00 \pm 0.10\%$ and total phenolic content 25.85 ± 0.00 mg GAE/g of dry weight. The Gac aril powder had the lightness of 58.59 ± 0.20 , redness of 25.39 ± 0.03 and yellowness of 27.58 ± 0.18 .

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INTRODUCTION

Gac fruit (*Momordica cochinchinensis* (Lour.) Spreng) is classified in the Cucurbitaceae belongs to melon family. Names in each country are used such as Phuck khow (Thailand), Moc Niet Tu (China), Bhat kerala (India), Mak kao (Laos) and Gac (Vietnam) (Kubola and Siriamornpun, 2011). The calling names in each locality of Thailand are Puk khow (Tak), Ma khow (Phrae) and Kheka Chain (Pattani) (Songsri, 2012). Gac fruit grows in many Asian countries (Vuong et al, 2006) such as Vietnam, India, Thailand, Laos and China. The edible portions are the seed coat (aril) and pulp in the maturation stage and the unripe fruit (green). The texture of Gac fruit is similar to papaya while the seed coat is similar to bitter melon (*Momordica charantia* L.). Gac fruit is a vegetable having different types of carotenoid phytochemicals. The aril contains mostly lycopene and the other parts have higher beta carotene content. Lycopene has antioxidant property for protecting different types of cancer such as gastrointestinal cancer and lung cancer (Kubola and Siriamornpun, 2011) and β -carotene can be converted to pro-vitamin A (Azqueta and Collins, 2012). Apart from the health benefit, the color of carotenoids can also be used as a red pigment in the natural food color (Scotter, 2014). The color and function of lycopene can easily be lost due to high temperature (100°C) and long duration time of processing (Lee and Chen, 2002). The decay of carotenoids is also due to oxidation, light, temperature and oxygen.

Freeze drying known as Lyophilization or Cryodesiccation is dehydration by the freezing process, that changes water to ice. The reduction of the pressure is to allow the ice crystals to sublimate (Rungsardthong, 2004). The freeze-dried food products show minimization of flavor and aroma losses, but maximization of nutrient retention and porous structure (Ratti, 2013).

Therefore, the research was focused on the factors affecting stability of lycopene and β -carotene from freeze-dried Gac aril powder.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu's phenol reagent and gallic acid were purchased from Fluka (Buchs, Switzerland). DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Aldrich (Steinheim, Germany). Acetone and n-hexane were purchased from Grec (New Zealand). Anhydrous sodium carbonate was purchased from Merck (Darmstadt, Germany). Maltodextrin 10 DE was purchased from Phacobic (Thailand). All other chemicals and solvents in this study were of analytical grade.

Particle size determination

Fresh Gac fruits were purchased from Nakhon Pathom province. The fruits were chosen on the fully ripe stage which appeared in the orange-red color. The fruits were cleaned and separated into pulp, seed and aril part. The aril was blended with different concentrations of maltodextrin DE 10 (0, 5, 10 and 15%, w/w) and then freeze-drying process was carried out at a condenser temperature of -20°C and pressure of 250 Pa for 48 h, in a freeze drier. The yield of aril powder was calculated. The freeze-dried arils (10 g) were grounded into powder, kept in non-vacuum-packed into laminated pouches and stored at 4°C and room temperature (27-30°C) until used. The samples were periodically withdrawn for 5 days for determination of lycopene, β -carotene and total phenolic content, antioxidant activity and color.

Determination of lycopene and β -carotene content

The analysis of lycopene and β -carotene were modified from Bhumsaidon and Chamchong (2016). One gram of Gac aril was put in a test tube. Then 10 ml of the mixed solvent of acetone and n-hexane in the ratio 1:3 (volume per volume) was added and homogenized using a homogenizer at 2900 rpm for 15 min and then subjected to test the capability of the absorption range of the UV-visible spectrophotometer. (Hitachi, U-2900, Japan) The light absorption wavelength values (A) at 453, 505, 663 and 645 nm were recorded for the determination of the lycopene and β -carotene contents in each sample. The lycopene and β -carotene contents were calculated according to equations (1) and (2) and reported based on 100 g of fresh Gac aril (FW).

$$\text{Lycopene (mg/100 ml)} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453} \quad (1)$$

$$\beta\text{-carotene (mg/100 ml)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453} \quad (2)$$

Determination of radical scavenging activity (DPPH assay)

The DPPH assay was carried out by investigating the ability to scavenge the DPPH[•] free radical. The DPPH[•] has an intense violet color with a maximum absorbance at 517 nm. In this study, the assay was modified from the method of Jang and Kim (2014). Using 96-well micro plate, reaction mixtures containing 35 μ l of sample and 200 μ l of 1N DPPH (prepared in 95% ethanol) were placed for 30 min at room temperature. The scavenging activity was calculated, according to following formula (3).

$$\% \text{ Radical scavenging} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100 \quad (3)$$

Determination of total phenolic content assay

The total phenolic content of the extracts was measured using Folin Ciocalteu reagent (FCR) method as described by Chuyen et al. (2017). The 96 - well microplate reaction containing 35 μ l of sample and 115 μ l of 10% FCR solution was kept in a dark place for 5 min. After that, 90 μ l of 7.5% Na₂CO₃ was added to each 96-well and the solution was mixed well and left for 60 min in the dark at room temperature. The absorbance of test sample was measured at 760 nm. The total phenolic content was determined by applying standard gallic acid calibration curve and expressed in mg of gallic acid equivalents (GAE)/g dried sample

Color measurement

The color of the Gac aril powder was measured using a Hunter Lab (ColorQuest XE, UK) calibrated with a white and black standard tile. The results were expressed as color values of L* (lightness), a* (redness and greenness) and b* (blueness and yellowness). Chroma, indicating color intensity, was calculated by formula (4). The hue angle (H°) was calculated, according to following formula (5). The hue angle values varied from 0° (pure red color), 90° (pure yellow color), 180° (pure green color) to 270° (pure blue color). Total color difference between two samples was calculated by the formula (6) (Tuyen, Nguyen and Roach (2010).

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} \quad (4)$$

$$H^{\circ} = \arctan(b^*/a^*) \quad (5)$$

$$\text{Total color difference } (\Delta E) = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

Statistical analysis

Data were expressed as means \pm standard deviation. The data were also subjected to analysis of variance (ANOVA) and Duncan's multiple range tests using SPSS 23.0 for Windows. The significance level of $p < 0.05$ was considered significantly different.

RESULTS AND DISCUSSION

Yield of Gac aril powders

The Gac aril powder using four different ratios (0%, 5%, 10% and 15% (w/v)) of commercial maltodextrin prior to freeze drying were prepared. Results showed the highest yield (32.39%) of freeze-dried aril powders were obtained when 15% maltodextrin was used (Table 1.). This is also supported by Pankeaw *et al.* (2016) who reported that resistant maltodextrin showed little difference in the yield of Gac aril powder mix 15% maltodextrin from spray drying.

Table 1. Yield of Gac aril powders from freeze drying

Sample	Yield (%)
Aril + 0% Maltodextrin	17.73
Aril + 5% Maltodextrin	20.41
Aril + 10% Maltodextrin	28.95
Aril + 15% Maltodextrin	32.39

Determination of lycopene and β -carotene content

The changes of lycopene and β -carotene content of freeze-dried powder stored at 4°C and room temperature (27-30°C) for 30 days are shown in Fig 1. The content of lycopene and β -carotene with no maltodextrin stored at 4°C had distinctly higher than those in the other conditions. The lycopene content in the freeze-dried Gac aril powder stored at 4°C and room temperature were 63.00 \pm 0.00 mg/100 g DW and 37.81 \pm 0.32 mg/100 DW, respectively (Fig 1.). The β -carotene content in the freeze-dried Gac aril powder during 30 days of storage at 4°C and room temperature was in the content of 18.00 \pm 0.01 mg/100 g DW and 17.52 \pm 0.00 mg/100 g DW, respectively (Fig 1.). For the lycopene and β -carotene content in both the temperature storage and concentration of maltodextrin used, significant differences were observed, regardless of storage temperature at 4°C and room temperature. These results indicated that the storage temperature (4°C) or room temperature and concentration of maltodextrin have effect on the loss of the lycopene and β -carotene content in the powder. Mai *et al.* (2013) studied the impact of limited drying on carotenoids content in the freeze-dried Gac aril powder. The results showed that the total carotenoid in the freeze-dried powder remained 82%. Based on the results of this study, the lycopene and β -carotene content in the low temperature storage powder was significantly higher than that in the high temperature storage, which indicated that the low temperature storage is more beneficial for the retention of lycopene and β -carotene content compared to the high temperature storage.

Radical scavenging activity (DPPH assay)

The DPPH is stable and is widely used to evaluate the radical scavenging activity of antioxidant compounds. The antioxidant is able to act as a donor of hydrogen atom and transforms to DPPH radical. The antioxidant activity of the all freeze-dried Gac aril powders having different ratios of commercial maltodextrin used and different temperature storage are presented in Fig 2. The results showed that the radical scavenging activity capacity of all the samples decreased during 30 days of storage. The decrease trend in DPPH radical content of the samples stored at the temperature of 4°C and room temperature was similar. The percentage of DPPH radicals of Gac aril powder stored at 4°C and room temperature was in the content of 32.50 \pm 0.00% and 25.80 \pm 0.00%, respectively. Results showed that the storage condition (both temperature and duration time) affected the DPPH radical scavenging activities. The antioxidant activity can be presented in IC50 value. Kubola and Siriamornpun. (2011) reported that the antioxidant activity was dependent on the actual composition of the Gac aril having IC 50 of 3.66 mg/ml.

Total Phenolic content

Phenolic compounds are widely distributed in plants and have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health (Govindarajan *et al.*, 2007). The phenolic acid component (TPC) of freeze-dried Gac aril powder is shown in Fig 2. The TPC content in the freeze-dried Gac aril powder stored at 4°C and room temperature for 30 days was in the content of 14.88 \pm 0.00 mg GAE/g DW and 8.62 \pm 0.00 mg GAE/g DW, respectively. Kubola and Siriamornpun. (2011) studied the phytochemicals and antioxidant activity of Gac fruit and reported the TPC results of 4.29 \pm 0.15 mg GAE/g. The results were different slightly because of different origin and different genotype used. Gac fruit has a more than 13 genotypes in Thailand (Nanta *et al.*, 2016).

Colorimetry

The color values of L*, a* and b* of the fresh Gac aril were 34.73 \pm 0.61-35.39 \pm 0.79, 39.20 \pm 0.25-40.21 \pm 0.79 and 33.27 \pm 0.80-34.68 \pm 0.47, respectively. The color values of the freeze-dried Gac powder stored at 4°C and room temperature for 30 days were 56.57 \pm 0.43; 66.39 \pm 0.10, 25.41 \pm 0.10; 16.98 \pm 0.15 and 25.54 \pm 0.56; 20.03 \pm 0.24, respectively. It is assumed that this red color is generated by the presence of carotenoid, mainly lycopene and β -carotene in the composition of the fruit (Mai *et al.*, 2013). The statistic results showed that the fresh Gac aril and Gac aril powder had significantly differences of L*, a*, b*, ΔE , Chroma and H° values for a given storage temperature during 30 days. (Fig 3 and Fig 4). The minimal total color change obtained if the low temperature storage and maltodextrin. were used.

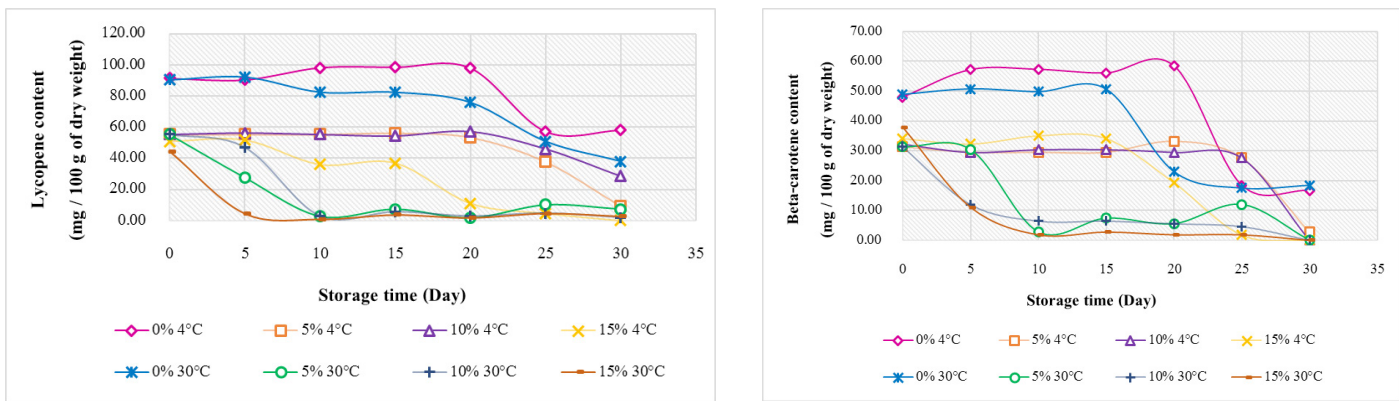


Figure 1. The changes of lycopene and β -carotene content in freeze-dried powder on 30 days storage at 4°C and room temperature(27-30°C).

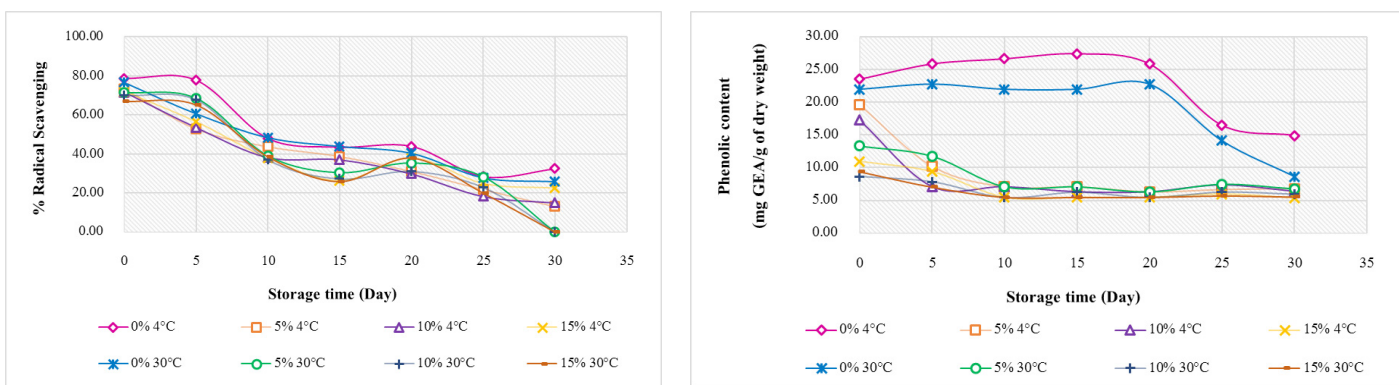


Figure 2. The changes of radical scavenging activity and total phenolic content in freeze-dried powder on 30 days storage at 4°C and room temperature (27-30°C).

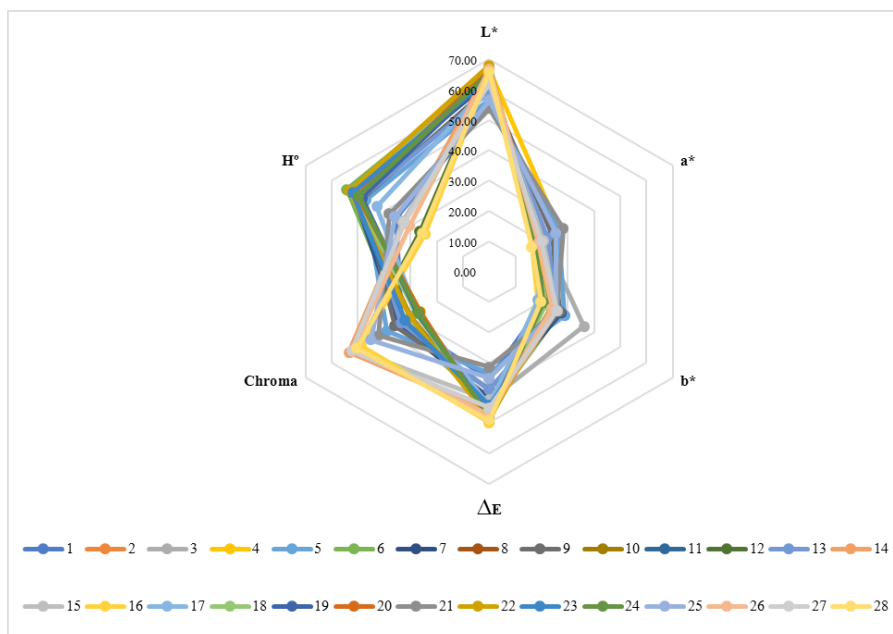


Figure 3. The color values of L*, a*, b*, ΔE , Chroma and H° values for a given freeze-dried powder storage at 4°C temperature during 30 days.

- 1 : Day 0+0% maltodextrin 2 : Day 0+5% maltodextrin 3 : Day 0+10% maltodextrin 4 : Day 0+15% maltodextrin
- 5 : Day 5+0% maltodextrin 6 : Day 5+5% maltodextrin 7 : Day 5+10% maltodextrin 8 : Day 5+15% maltodextrin
- 9 : Day 10+0% maltodextrin 10 : Day 10+5% maltodextrin 11 : Day 10+10% maltodextrin 12 : Day 10+15% maltodextrin
- 13 : Day 15+0% maltodextrin 14 : Day 15+5% maltodextrin 15 : Day 15+10% maltodextrin 16 : Day 15+15% maltodextrin
- 17 : Day 20+0% maltodextrin 18 : Day 20+5% maltodextrin 19 : Day 20+10% maltodextrin 20 : Day 20+15% maltodextrin
- 21 : Day 25+0% maltodextrin 22 : Day 25+5% maltodextrin 23 : Day 25+10% maltodextrin 24 : Day 25+15% maltodextrin
- 25 : Day 30+0% maltodextrin 26 : Day 30+5% maltodextrin 27 : Day 30+10% maltodextrin 28 : Day 30+15% maltodextrin

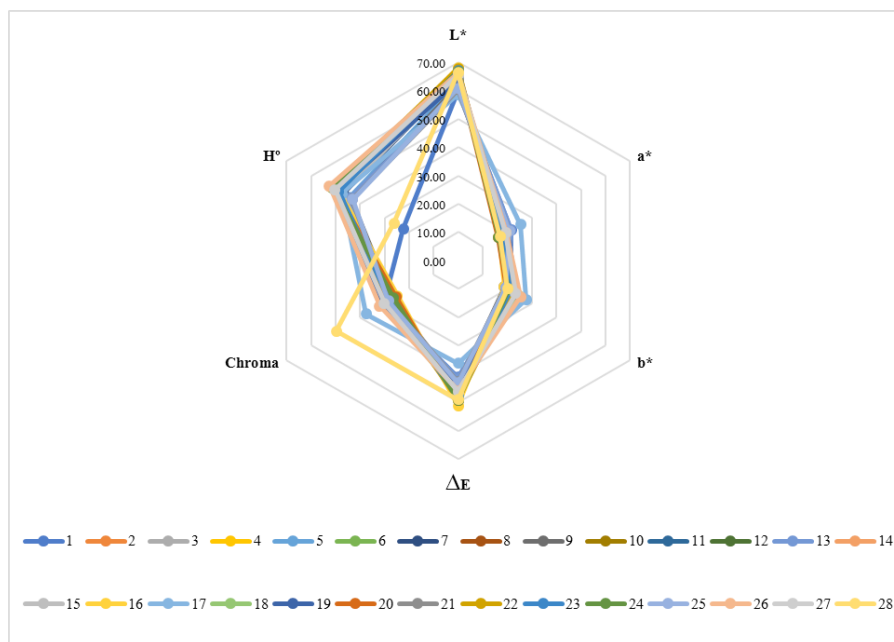


Figure 4. The color values of L*, a*, b*, ΔE, Chroma and H° values for a given freeze-dried powder storage at room temperature (27-30°C) during 30 days.

- | | | | |
|-----------------------------|-----------------------------|------------------------------|------------------------------|
| 1 : Day 0+0% maltodextrin | 2 : Day 0+5% maltodextrin | 3 : Day 0+10% maltodextrin | 4 : Day 0+15% maltodextrin |
| 5 : Day 5+0% maltodextrin | 6 : Day 5+5% maltodextrin | 7 : Day 5+10% maltodextrin | 8 : Day 5+15% maltodextrin |
| 9 : Day 10+0% maltodextrin | 10 : Day 10+5% maltodextrin | 11 : Day 10+10% maltodextrin | 12 : Day 10+15% maltodextrin |
| 13 : Day 15+0% maltodextrin | 14 : Day 15+5% maltodextrin | 15 : Day 15+10% maltodextrin | 16 : Day 15+15% maltodextrin |
| 17 : Day 20+0% maltodextrin | 18 : Day 20+5% maltodextrin | 19 : Day 20+10% maltodextrin | 20 : Day 20+15% maltodextrin |
| 21 : Day 25+0% maltodextrin | 22 : Day 25+5% maltodextrin | 23 : Day 25+10% maltodextrin | 24 : Day 25+15% maltodextrin |
| 25 : Day 30+0% maltodextrin | 26 : Day 30+5% maltodextrin | 27 : Day 30+10% maltodextrin | 28 : Day 30+15% maltodextrin |

CONCLUSIONS

The quality of freeze dried Gac aril powder was affected by storage temperature (4°C and room temperature), duration of time (0-30 days) and concentration of maltodextrin used on lycopene, β-carotene and qualitative characteristics. Based on the study, the freeze-dried Gac with 0% maltodextrin stored at low temperature (4°C) for 20 days yielded the highest values of lycopene, β-carotene, DPPH radical scavenging activities and color values.

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